(ISSN Online: 3006-1113) Full Paper

Libyan Journal of Medical and Applied Sciences

Journal homepage: https://ljmas.com/index.php/journal/index

A RESEARCH ARTICLE

Standardized Punica granatum Peel Extract Attenuates Adenine-Induced Chronic Kidney Disease in Rats: Effects on Renal Function, Oxidative Stress, Renal Histology, and Colon Morphology

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Article Information

Article history:

Received on: 09/Mar/2026
Revised on: 02/Jun/2026
Accepted on:04/May/2026
Published on: 06/Jun/2026

Keywords:

Punica granatum;
chronic kidney disease;
oxidative stress;
renal histology;
colon histology.

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Abstract

Chronic kidney disease (CKD) is defined by advancing renal impairment, structural kidney damage, oxidative dysregulation, and systemic metabolic anomalies. Adenine feeding is commonly employed to replicate CKD-like renal dysfunction and tubulointerstitial injury in rodents. This study assessed the efficacy of standardized Punica granatum peel extract (PPE) in alleviating adenine-induced chronic kidney disease (CKD) in rats. Methods: Rats were divided into six groups: normal control (NC), PPE-only, CKD control, CKD with low-dose PPE (CKD + PPE-L), CKD with high-dose PPE (CKD + PPE-H), and CKD with AST-120 reference treatment. The parameters examined included final body weight, food consumption, serum creatinine, serum urea, serum uric acid, renal malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), renal histology, and colon morphology. Results: CKD controls had significant increase in serum creatinine, urea and uric acid to 2.72 ± 0.35 mg/dL, 168.4 ± 18.7 mg/dL and 6.8 ± 0.9 mg/dL respectively compared to 0.43 ± 0.06 mg/dL, 37.2 ± 5.1 mg/dL and 1.3 ± 0.3 mg/dL in normal controls. High dose PPE improved these abnormalities with serum creatinine of 1.31 ± 0.22 mg/dL, urea of 90.6 ± 12.5 mg/dL and uric acid of 3.3 ± 0.5 mg/dL. Adenine also increased renal MDA, decreased GSH, SOD and CAT. High-dose PPE reduced MDA and normalized GSH, SOD and CAT. CKD controls had severe renal and colonic injury by histology with total renal and colonic injury scores of 28 and 21, respectively. High dose PPE significantly reduced these scores to 6 and 5 respectively. Conclusion: These study findings suggest that PPE may exert nephroprotective activity via multi-target antioxidant, anti-inflammatory, epithelial-protective and gut–kidney axis-related mechanisms. However, no direct inhibition of adenine uptake or 2,8-dihydroxyadenine crystal deposition was shown and requires further mechanistic investigation.

1. Introduction

Chronic kidney disease is a progressive condition characterized by enduring irregularities in kidney structure or function, with significant health ramifications. It is clinically significant because deteriorating kidney function leads to metabolic, cardiovascular, nutritional, and inflammatory effects that transcend the kidney itself [1,2]. The worldwide burden of chronic kidney disease (CKD) has significantly risen, making it a major cause of early illness and death, especially among individuals with diabetes, hypertension, older age, and restricted access to early screening [3].

Experimental CKD models are indispensable for mechanistic and therapeutic research as they facilitate the integration of functional, biochemical, and histological endpoints in controlled environments. Adenine-induced chronic kidney disease (CKD) is a frequently employed non-surgical model. After administration, adenine is transformed into poorly soluble metabolites that accumulate in renal tubules, resulting in tubular obstruction, tubulointerstitial injury, renal dysfunction, and progressive structural alterations akin to significant features of human chronic kidney disease [4,5].

The progression of chronic kidney disease (CKD) is caused by a combination of losing nephrons, damaging tubular epithelial cells, failing to repair damage, poor blood flow, oxidative stress, and renal fibrosis[6]. Oxidative stress is particularly pertinent as chronic kidney disease (CKD) is linked to elevated reactive oxygen species production and compromised antioxidant defenses, fostering conditions conducive to lipid peroxidation, tubular injury, vascular dysfunction, and additional nephron damage [7,8].

DOI: https://doi.org/10.64943/ljmas.2026.040211

The gut-kidney axis has attracted attention as a biologically plausible connection between intestinal dysfunction and the progression of renal disease. Chronic kidney disease (CKD) changes the microbial ecosystem in the gut and weakens the epithelial barrier. Uremic conditions can also damage structures that are connected by tight junctions in the colon and small intestine [9,10]. As renal clearance decreases, gut-derived protein-bound uremic toxins, such as indoxyl sulfate and p-cresyl sulfate, accumulate and have been linked to oxidative damage, vascular dysfunction, and negative outcomes in chronic kidney disease [11,12]. In this case, AST-120 has been used as an oral intestinal adsorbent that targets toxin precursors. However, the results of clinical trials have been mixed and should be taken with a grain of salt [13].

Punica granatum L. peel is a polyphenol-rich plant material that has attracted pharmacological interest because the peel contains abundant hydrolysable tannins, punicalagins, ellagic acid derivatives, flavonoids, and other phenolic constituents. These compounds are closely associated with antioxidant capacity and may contribute to tissue protection in oxidative injury models [14–16]. Importantly, the biological interpretation of plant extracts depends on rigorous standardization. Moisture content, ash values, extractive values, phytochemical screening, total phenolics, total flavonoids, and chromatographic markers such as punicalagin and ellagic acid are commonly used to improve reproducibility and quality control in pharmacognostic research [17–19]. The study aimed to evaluate punica granatum peel extract attenuates adenine-induced chronic kidney disease and the effects on renal function, oxidative stress, renal histology, and colon morphology in rat's model.

2. Materials and Methods

2.1. *Plant material and authentication*

Fresh fruits of Punica granatum L. were procured at mature stage from a reliable local source. The peels were separated from the edible arils and washed with distilled water to remove adhering impurities and examined to exclude damaged or contaminated material. The shade was dried under controlled laboratory conditions, protected from direct sunlight to minimise degradation of phenolic constituents, and the peel material was ground to a coarse powder in a clean mechanical grinder. The powdered material was stored at room temperature in airtight containers resistant to light until extraction.

2.1. Extract preparation

The powdered peel material was extracted with 70% ethanol in distilled water at a ratio of plant material to solvent of 1:10 w/v. The extraction was performed by maceration for 72 hours at room temperature with intermittent agitation. To preserve the stability of polyphenolic compounds, the extraction vessel was tightly closed and protected from direct light during the extraction period. Hydroethanolic solvents are commonly used to extract pomegranate peel due to their efficient recovery of phenolics, flavonoids, hydrolysable tannins, punicalagin, ellagic acid and related antioxidant constituents [17,19,20].

After extraction, the mixture was filtered through clean muslin cloth and subsequently through Whatman No. 1 filter paper to remove insoluble plant residue. The filtrate was concentrated by rotary evaporation under reduced pressure at 40–45°C until the solvent was removed and a concentrated semisolid extract was obtained. Rotary evaporation under reduced pressure and moderate temperature reduces thermal degradation of heat-sensitive phytochemicals while allowing efficient solvent removal [18,19]. The concentrated extract was collected, placed into clean amber-colored containers and stored at 4°C until use.

The concentrated extract of Punica granatum peel was freshly suspended in 0.5% carboxymethyl cellulose as vehicle before administration. Punica granatum peel extract was administered orally via the gavage in the low-dose and high-dose treatment groups at 100 mg/kg and 200 mg/kg body weight, respectively [18,21].

2.2. Experimental animals

A total of 36 rats, 8–10 weeks of age and weighing 180–220 g at the beginning of the experiment, were randomly allocated into six experimental groups, with six rats in each group in this study. Animals were obtained from the institutional animal house and were clinically examined before inclusion to ensure that only healthy rats without visible signs of disease, injury, or abnormal behavior were enrolled. Male rats were selected to reduce physiological variability related to hormonal cycling and to provide a consistent experimental background for evaluating adenine-induced chronic kidney disease.

Chronic kidney disease was induced using dietary adenine, a well-established experimental approach for producing renal dysfunction and tubulointerstitial injury in rats. Briefly, adenine was thoroughly mixed with the standard powdered laboratory diet at a concentration of 0.75% w/w and administered to the CKD-designated groups for 4 consecutive weeks. The adenine-containing diet was freshly prepared at regular intervals and stored in airtight containers to maintain uniformity and prevent contamination. [4,5].

The animals were divided into six experimental groups, with six rats in each group. The normal control group (NC) received standard laboratory diet and vehicle only. The Punica granatum peel extract-only group (PPE) received standard laboratory diet and high-dose Punica granatum peel extract at 200 mg/kg body weight/day to evaluate the safety of the extract in healthy animals. The CKD control group received the adenine-containing diet and vehicle only. The CKD + PPE-L group received the adenine-containing diet together with low-dose Punica granatum peel extract at 100 mg/kg body weight/day. The CKD + PPE-H group received the adenine-containing diet together with high-dose Punica granatum peel extract at 200 mg/kg body weight/day. The CKD + AST-120 group received the adenine-containing diet together with AST-120 at 4 g/kg/day as a reference gut-directed intervention.

Punica granatum peel extract was freshly suspended in 0.5% carboxymethyl cellulose immediately before administration and given once daily by oral gavage throughout the 4-week experimental period. The same vehicle was administered to the NC and CKD control groups to ensure consistency among groups. AST-120 was administered orally at 4 g/kg/day, preferably by mixing it with the daily diet to maintain its intestinal adsorptive action. The inclusion of AST-120 provided a relevant comparator because it is an orally administered carbon adsorbent investigated for its ability to bind intestinal precursors of uremic toxins, particularly indole-derived compounds, thereby reducing the systemic burden of gut-derived uremic solutes such as indoxyl sulfate [13]. Body weight and food intake were monitored during the experiment, and the administered extract doses were adjusted according to the most recent body weight to maintain accurate dose delivery.

2.3. Sample collection

At the end of the experimental period, rats were fasted overnight with free access to water and then anaesthetized with intraperitoneal injection of ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg). Prior to the collection of terminal samples, the absence of pedal withdrawal and corneal reflexes confirmed adequate depth of anaesthesia. Blood was drawn by cardiac puncture using a sterile 5 ml syringe with a 23-gauge needle and transferred into plain gel-free tubes. Blood samples were allowed to clot at room temperature for 30 min, and then centrifuged at 3000 rpm for 10 min at 4 °C. The separated serum was transferred to clean Eppendorf tubes and stored at -20 °C until biochemical analysis of serum creatinine, urea and uric acid.

2.4. Biochemical and oxidative stress tests

Serum creatinine, urea and uric acid were measured using commercially available FUJIFILM diagnostic kits following the manufacturer’s instructions. Renal oxidative stress markers were measured in kidney tissue homogenates using commercially available SunLong ELISA assay kits according to the manufacturer’s instructions. Malondialdehyde, reduced glutathione, superoxide dismutase and catalase were determined as indices of lipid peroxidation and antioxidant defense status.

2.5. Histological examination

Kidney and colon specimens were described using hematoxylin and eosin (H&E)-stained sections. Renal histology focused on glomerular preservation or injury, Bowman's space, tubular dilation, epithelial degeneration or desquamation, tubular necrosis, tubular atrophy, casts, crystal-associated material or spaces, interstitial edema, vascular congestion, and sclerosis-like changes. Colon histology surface epithelium, crypt architecture, goblet cell density, lamina propria changes, epithelial erosion, edema, and inflammatory cell infiltration as a morphological feature. Standard histological processing included fixation, dehydration, clearing, paraffin embedding, microtomy, H&E staining, and blinded microscopic assessment when applicable [22].

2.6. Statistical analysis

GraphPad Prism version 9.0 was used for data analysis. Results are shown as mean ± SD. Normality of data was tested by Shapiro–Wilk test and homogeneity of variance was tested by Levene's test. Data with normal distribution were compared using one-way ANOVA with Tukey post hoc test. Where applicable, histological scores and non-normally distributed or ordinal data were analysed with Kruskal–Wallis test with Dunn's post hoc test. When variables were measured over time (e.g., body weight), repeated-measures ANOVA was used as appropriate. Statistical significance was accepted at $p < 0.05$. The significance symbols were defined as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. normal control group; #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ vs. CKD control group.

3. Results

3.1. Effects on general physiological and renal function parameters

Adenine administration produced a clear CKD-like phenotype. Compared with NC rats, CKD controls showed marked reduction in final body weight and food intake, increased kidney index, increased serum creatinine, urea, and uric acid. PPE treatment improved these abnormalities in a dose-dependent pattern. Low-dose PPE partially improved body weight, food intake, kidney index, whereas high-dose PPE produced greater correction across nearly all measured endpoints. The high-dose PPE group showed lower serum creatinine, urea, uric acid than the CKD control group and generally showed a stronger profile than the AST-120 reference group for several renal function parameters (Table 1).

Table 1. Effects of standardized Punica granatum peel extract on general physiological and renal function parameters.

Parameter	NC	PPE	CKD	CKD + PPE-L	CKD + PPE-H	CKD + AST-120
Final body weight (g)	285 ± 18	288 ± 16	218 ± 20***	242 ± 19**#	260 ± 17*##	254 ± 18*##
Food intake (g/day)	22.4 ± 1.8	22.1 ± 1.7	15.2 ± 2.0***	17.8 ± 1.9**#	19.6 ± 1.8*##	18.9 ± 1.6**##
Kidney index (mg/g body weight)	7.1 ± 0.5	7.0 ± 0.4	12.5 ± 1.3***	10.4 ± 1.1***#	8.9 ± 0.9**###	9.4 ± 1.0**##
Serum creatinine (mg/dL)	0.43 ± 0.06	0.42 ± 0.05	2.72 ± 0.35***	1.95 ± 0.28***##	1.31 ± 0.22***###	1.52 ± 0.25***###
Serum urea (mg/dL)	37.2 ± 5.1	36.5 ± 4.8	168.4 ± 18.7***	128.3 ± 15.4***##	90.6 ± 12.5***###	104.2 ± 14.3***###
Serum uric acid (mg/dL)	1.3 ± 0.3	1.2 ± 0.2	6.8 ± 0.9***	4.9 ± 0.7***##	3.3 ± 0.5**###	3.8 ± 0.6***###

Values are shown as mean ± SD. NC, normal control; PPE, Punica granatum peel extract; CKD, chronic kidney disease; PPE-L, low-dose PPE; PPE-H, high-dose PPE. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus NC; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ versus CKD.

3.2. Effects on renal oxidative stress and antioxidant defense

Renal oxidative stress was substantially altered in CKD controls. Adenine increased renal MDA and reduced GSH, SOD, and CAT compared with NC rats, indicating enhanced lipid peroxidation and weakened antioxidant defense. PPE attenuated this oxidative imbalance, again with a stronger response in the high-dose group. High-dose PPE reduced MDA toward control values and restored GSH, SOD, and CAT more effectively than low-dose PPE. AST-120 also improved oxidative stress

markers, but high-dose PPE showed numerically stronger restoration for MDA, GSH, SOD, and CAT (Table 2).

Table 2. Effects of standardized Punica granatum peel extract on renal oxidative stress and antioxidant defense.

Parameter	NC	PPE	CKD	CKD + PPE-L	CKD + PPE-H	CKD + AST-120
Renal MDA (nmol/mg protein)	2.4 ± 0.3	2.3 ± 0.3	6.8 ± 0.7***	5.0 ± 0.6***##	3.5 ± 0.5**###	3.9 ± 0.5**###
Renal GSH (µmol/g tissue)	8.1 ± 0.7	8.3 ± 0.6	4.0 ± 0.5***	5.3 ± 0.6***##	6.8 ± 0.7*###	6.3 ± 0.6**###
Renal SOD (U/mg protein)	13.2 ± 1.2	13.5 ± 1.1	6.9 ± 0.8***	8.9 ± 0.9***##	11.3 ± 1.0#	10.6 ± 0.9*###
Renal CAT (U/mg protein)	55.4 ± 5.2	56.1 ± 4.9	30.6 ± 4.1***	39.2 ± 4.8***##	48.6 ± 5.0#	45.3 ± 4.6*###

*Values are shown as mean ± SD. NC, normal control; PPE, Punica granatum peel extract; CKD, chronic kidney disease; PPE-L, low-dose PPE; PPE-H, high-dose PPE. *p < 0.05, **p < 0.01, ***p < 0.001 versus NC; #p < 0.05, ##p < 0.01, ###p < 0.001 versus CKD.*

3.3. Renal histopathological findings

Representative renal H&E descriptions indicated preserved renal architecture in NC rats, with intact glomeruli, clear Bowman's spaces, and normal renal tubules. PPE-only sections were described as near-normal, suggesting no evident extract-related renal toxicity. CKD controls showed severe renal injury, including tubular dilation, epithelial degeneration and desquamation, tubular necrosis and atrophy, intratubular casts, crystal-associated material or spaces, interstitial edema, glomerular injury, widened Bowman's space, sclerosis-like changes, and vascular congestion. Low-dose PPE partially improved the renal architecture but residual tubular and interstitial injury persisted. High-dose PPE produced marked renal protection, with mostly preserved glomeruli and tubules and only minimal residual inflammatory infiltration as a histological feature. AST-120 produced moderate-to-marked renal protection with reduced tubular injury, casts, and inflammatory infiltration (Figure 1).

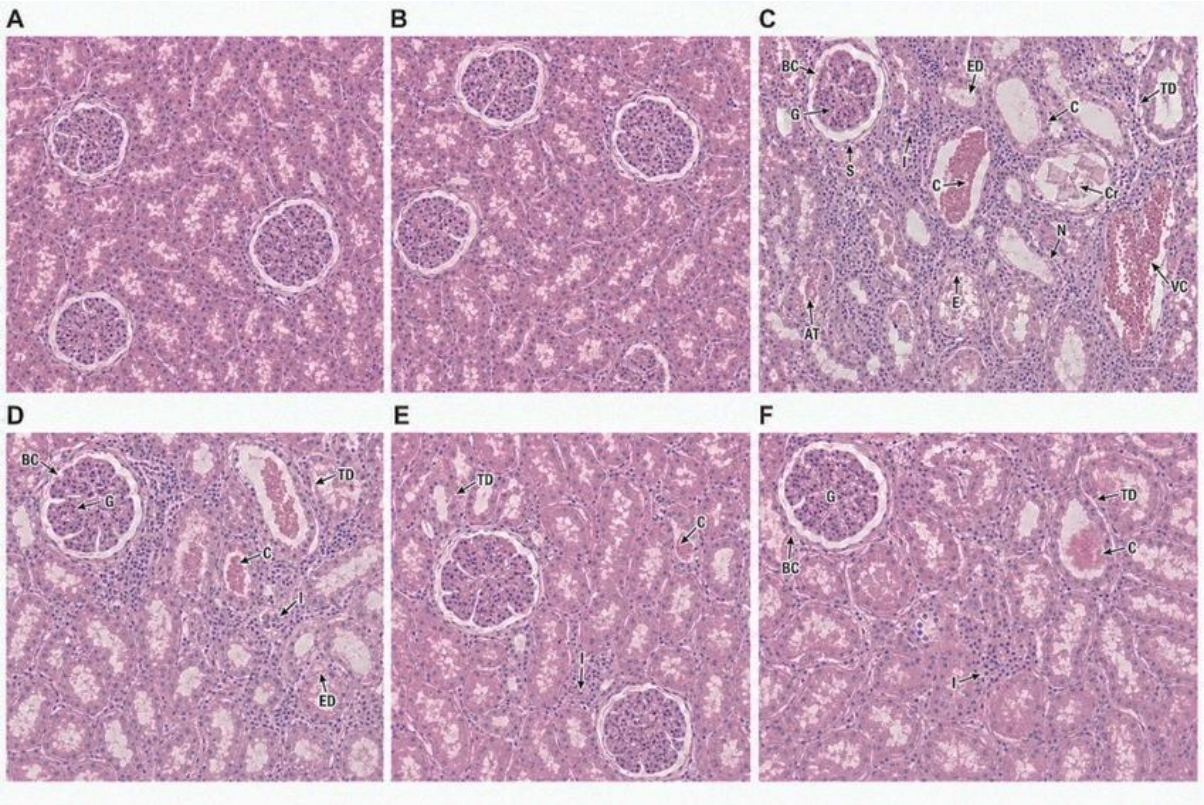

Figure 1. Representative H&E-stained kidney sections in adenine-induced CKD and PPE-treated rats.

showing renal histopathological changes in adenine-induced chronic kidney disease and the protective effect of standardized Punica granatum peel extract. (A) Normal control group showing preserved renal architecture, intact glomeruli, clear Bowman's spaces, and normal renal tubules. (B) Punica granatum peel extract-only group showing near-normal renal histology without evidence of extract-related toxicity. (C) Adenine-induced CKD control group showing severe renal injury, including tubular dilation, epithelial degeneration/desquamation, tubular necrosis, tubular atrophy, intratubular casts, crystal-associated spaces/material, interstitial edema, inflammatory cell infiltration as a morphological feature, glomerular injury, widened Bowman's space, sclerosis-like changes, and vascular congestion. (D) CKD + low-dose PPE group showing partial improvement with moderate residual tubular and interstitial injury. (E) CKD + high-dose PPE group showing marked renal protection with mostly preserved glomeruli and tubules and minimal residual inflammatory cell infiltration. (F) CKD + reference treatment group showing moderate-to-marked renal protection with reduced tubular injury, casts, and inflammatory cell infiltration. Abbreviations: AT, tubular atrophy; BC, widened Bowman's space; C, cast; CKD, chronic kidney disease; Cr, crystal-associated material/space; ED, epithelial degeneration/desquamation; G, glomerular injury; I, inflammatory cell infiltration; N, necrosis; PPE, Punica granatum peel extract; S, sclerosis-like change; TD, tubular dilation; VC, vascular congestion. H&E stain, original magnification x400.

Table 3. semi-quantitative renal histopathological scoring in adenine-induced CKD and PPE-treated rats

Group	Tubular dilation	Epithelial degeneration / desquamation	Tubular necrosis	Tubular atrophy	Casts / crystal-associated spaces	Interstitial edema	Inflammatory cell infiltration	Glomerular / Bowman's space injury	Total renal injury score
Normal control	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–1)^{a}
PPE-only	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–1)^{a}	0 (0–0)^{a}	1 (0–1)^{a}
CKD control	4 (3–4)^{b}	4 (3–4)^{b}	3 (3–4)^{b}	3 (3–4)^{b}	4 (3–4)^{b}	3 (3–4)^{b}	4 (3–4)^{b}	3 (2–3)^{b}	28 (25–30)^{b}
CKD + PPE 100 mg/kg	2 (2–3)^{c}	2 (2–3)^{c}	2 (1–2)^{c}	2 (1–3)^{c}	2 (2–3)^{c}	2 (1–2)^{c}	2 (2–3)^{c}	1 (1–2)^{c}	15 (13–18)^{c}
CKD + PPE 200 mg/kg	1 (0–1)^{d}	1 (0–1)^{d}	0 (0–1)^{d}	1 (0–1)^{d}	1 (0–1)^{d}	1 (0–1)^{d}	1 (0–1)^{d}	0 (0–1)^{d}	6 (4–8)^{d}
CKD + AST-120 4 g/kg	1 (1–2)^{de}	1 (1–2)^{de}	1 (1–2)^{de}	1 (1–2)^{de}	2 (1–2)^{ce}	1 (1–2)^{de}	2 (1–2)^{ce}	1 (0–1)^{d}	10 (8–13)^{e}

Table annotation. Values are presented as median (interquartile range). Renal histopathological lesions were scored semi-quantitatively on a 0–4 ordinal scale: 0 = absent/normal, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe. Groups sharing at least one superscript letter are not significantly different.

3.4. Colon histopathological findings

Colon H&E descriptions showed intact surface epithelium, regular crypt architecture, abundant goblet cells, and normal lamina propria in NC rats. PPE-only sections were near-normal, without evidence of extract-related toxicity. CKD controls showed marked mucosal injury, including epithelial erosion, crypt architectural distortion, reduced goblet cell density, mucosal edema, expanded lamina propria, and inflammatory cell infiltration as a morphological feature. Low-dose PPE partially preserved mucosal architecture, with improved crypt organization and goblet cell preservation but residual epithelial injury, edema, and inflammatory infiltration. High-dose PPE produced substantial mucosal protection, with improved goblet cell density, preserved mucosal architecture, and minimal residual inflammatory changes. AST-120 showed moderate-to-marked mucosal improvement with reduced injury and improved crypt preservation (Figure 2).

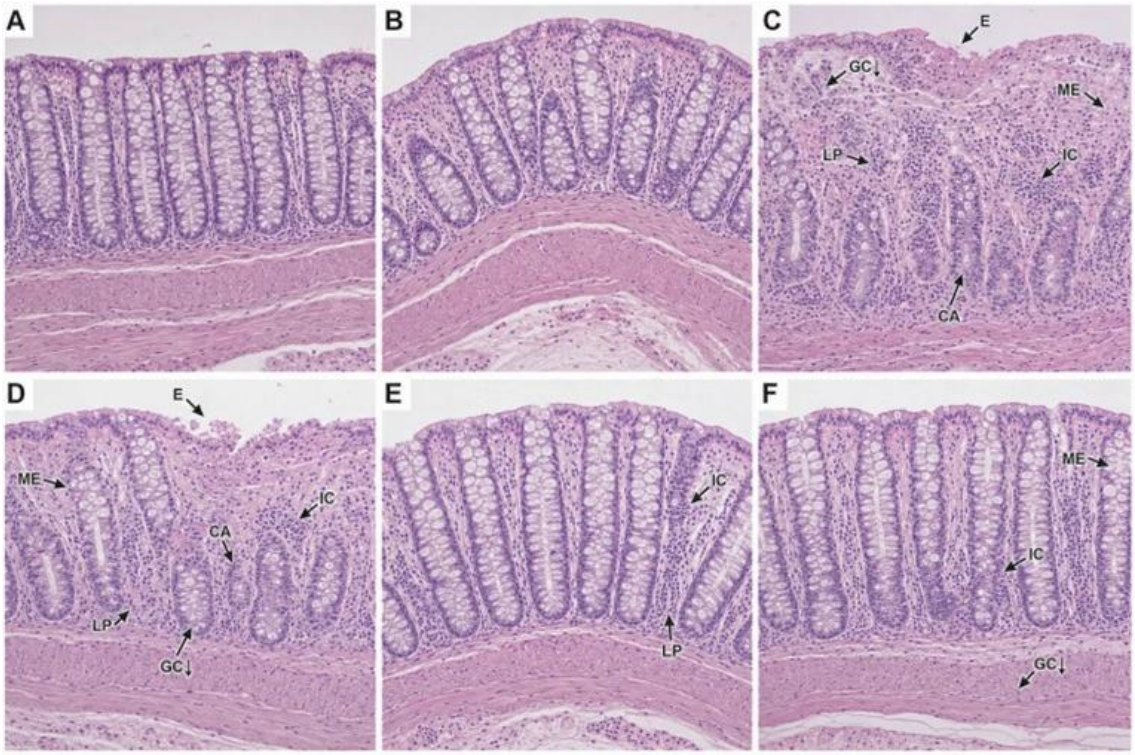

Figure 2. Representative H&E-stained colon sections in adenine-induced CKD and PPE-treated rats.

Normal control group showing intact colonic surface epithelium, regular crypt architecture, abundant goblet cells, and normal lamina propria. (B) Punica granatum peel extract-only group showing near-normal colonic mucosa without evidence of extract-related toxicity. (C) Adenine-induced CKD control group showing marked mucosal injury characterized by epithelial erosion, crypt architectural distortion, reduced goblet cell density, mucosal edema, expanded lamina propria, and inflammatory cell infiltration as a morphological feature. (D) CKD + low-dose PPE group showing partial mucosal protection with improved crypt organization and goblet cell preservation, but with residual epithelial injury, edema, and inflammatory infiltration. (E) CKD + high-dose PPE group showing substantial mucosal protection with preserved mucosal architecture, improved goblet cell density, and minimal inflammatory changes. (F) CKD + reference treatment group showing moderate-to-marked improvement with reduced mucosal injury and improved crypt preservation. Abbreviations: CA, crypt architectural distortion; CKD, chronic kidney disease; E, epithelial erosion; GC↓, reduced goblet cells; IC, inflammatory cell infiltration; LP, expanded lamina propria; ME, mucosal edema; PPE, Punica granatum peel extract. H&E stain, approximate original magnification x400.

Table 4. semi-quantitative colonic histopathological scoring in adenine-induced CKD and PPE-treated rats

Group	Epithelial erosion	Crypt architectural distortion	Goblet cell depletion	Mucosal edema	Lamina propria expansion	Inflammatory cell infiltration	Total colonic injury score
Normal control	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–1)^{a}	0 (0–1)^{a}
PPE-only	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–0)^{a}	0 (0–1)^{a}	1 (0–1)^{a}
CKD control	3 (3–4)^{b}	4 (3–4)^{b}	3 (3–4)^{b}	3 (3–4)^{b}	3 (3–4)^{b}	4 (3–4)^{b}	21 (19–23)^{b}
CKD + PPE 100 mg/kg	2 (1–2)^{c}	2 (2–3)^{c}	2 (1–2)^{c}	2 (1–2)^{c}	2 (1–2)^{c}	2 (2–3)^{c}	12 (10–15)^{c}
CKD + PPE 200 mg/kg	1 (0–1)^{d}	1 (0–1)^{d}	1 (0–1)^{d}	1 (0–1)^{d}	1 (0–1)^{d}	1 (0–1)^{d}	5 (3–7)^{d}
CKD + AST-120 4 g/kg	1 (1–2)^{de}	2 (1–2)^{ce}	1 (1–2)^{de}	1 (1–2)^{de}	1 (1–2)^{de}	2 (1–2)^{ce}	9 (7–11)^{e}

Values are presented as median (interquartile range). Colonic histopathological lesions were scored semi-quantitatively on a 0–4 ordinal scale: 0 = absent/normal, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe. Goblet cell depletion was scored as 0 = normal goblet cell density and 4 = marked depletion. Different superscript letters within the same column indicate statistically significant differences between groups

4. Discussion

The results obtained demonstrate that the administration of adenine was successful in inducing a CKD-like phenotype in rats. CKD controls had reduced body weight, reduced food intake, increased kidney function parameters. This pattern is consistent with the known adenine model, in which intratubular deposition of adenine metabolites causes tubular obstruction, tubulointerstitial injury, and progressive renal dysfunction [4,5].

AST-120 also improved a few endpoints, which makes sense from a biological point of view since it is an intestinal adsorbent that has been studied in CKD. However, high-dose PPE showed stronger improvements in a few renal function and oxidative markers in these results [13].

The greater improvement with PPE at 200 mg/kg compared to AST-120 at 4 g/kg should be interpreted in terms of pharmacological mechanism, not the direct comparison of administered mass. A crude botanical extract and a carbonaceous intestinal adsorbent are not comparable interventions in terms of dose. AST-120 is a non-absorbed oral spherical carbon adsorbent that mainly works in the gastrointestinal tract by binding uremic toxin precursors, especially indole, to reduce the systemic generation and accumulation of indoxyl sulphate and other protein-bound uremic toxins [23,24].

On the other hand, pomegranate peel extract is a polyphenol-rich preparation containing biologically active compounds, such as punicalagin, ellagic acid, gallic acid, punicalin, catechins and other hydrolysable tannins and flavonoids [25]. These compounds may affect renal injury through several mechanisms, not limited to intestinal adsorption. Experimental evidence supports the ability of pomegranate rind extract to ameliorate the cisplatin-induced acute kidney injury through reduction of oxidative stress, inflammation and apoptosis [26]. Furthermore, punicalagin, the major pomegranate ellagitannin, has been reported to protect against cisplatin-induced nephrotoxicity via attenuation of oxidative stress, inflammatory response and apoptosis, and upregulation of Nrf2-associated antioxidant defenses [27]. Ellagic acid also exhibited reno protective activity in models of cisplatin-induced nephrotoxicity including reduction in creatinine, urea, oxidative stress and renal histopathological injury [28].

This mechanistic difference may account for the more significant improvement of serum creatinine, urea, and renal MDA by PPE at 200 mg/kg than AST-120 in the present study. AST-120 indirectly lowers oxidative and inflammatory burden by limiting precursors of gut-derived uremic toxins but does not provide renal tissue with direct antioxidant or anti-inflammatory bioactives. In contrast, PPE may have a dual local-systemic effect. Polyphenols could locally modulate the intestinal environment and reduce oxidative or inflammatory signals related to gut–kidney axis dysfunction. Pomegranate ellagitannins can be hydrolysed in the intestine and metabolised by gut microbiota to ellagic acid and urolithin metabolites systemically, which have been detected in plasma and urine after pomegranate intake [29].

Oxidative stress seems to be a key part of the protective pattern seen in this study. Adenine-induced CKD elevated renal MDA levels while decreasing GSH, SOD, and CAT levels, signifying increased lipid peroxidation and compromised antioxidant defenses. This corroborates the prevailing perspective that oxidative imbalance is a persistent characteristic of chronic kidney disease (CKD) and may exacerbate tubular injury, vascular dysfunction, and tissue damage [9,10]. PPE changed this trend by lowering MDA and bringing back GSH, SOD, and CAT [14–16].

The renal histological descriptions corroborate the biochemical results. Sections from CKD showed a lot of damage, including severe tubular dilation, epithelial degeneration and desquamation, necrosis, atrophy, casts, crystal-associated spaces/material, glomerular injury, widened Bowman's space, sclerosis-like changes, edema, and vascular congestion. The colon findings offer morphological evidence for a gut-kidney axis component, although the interpretation must adhere to the constraints of H&E morphology. Histologically, CKD controls showed epithelial erosion, crypt distortion, goblet cell depletion, mucosal edema, lamina propria expansion, and inflammatory cell infiltration. These results align with the intestinal barrier disruption associated with CKD documented in both experimental and clinical studies [9,10].

Adenine-induced CKD is mainly initiated by conversion of excess adenine into poorly soluble 2,8-dihydroxyadenine, which deposits within renal tubules and causes obstruction, epithelial injury, inflammation, and tubulointerstitial fibrosis [30,31]. Therefore, the improved colon morphology

observed in PPE-treated groups should not be interpreted as proof that PPE directly blocked intestinal adenine absorption or prevented renal crystal deposition, because these mechanisms were not measured in the present study. A more balanced explanation is that PPE may have reduced the severity of renal injury after crystal formation through its polyphenol-mediated antioxidant, anti-inflammatory, and epithelial-protective effects. In addition, preservation of colonic mucosal integrity may have limited gut barrier dysfunction, endotoxin translocation, dysbiosis-related inflammation, and gut-derived uremic toxin burden, thereby reducing systemic inflammatory amplification through the gut–kidney axis [30,32]. Although partial inhibition of purine oxidation by PPE constituents remains a plausible additional mechanism [23,32], this remain a hypothesis until renal crystal burden, urinary 2,8-dihydroxyadenine, adenine absorption, or xanthine oxidoreductase activity are directly assessed.

5. Conclusion

Standardized Punica granatum peel extract improved adenine-induced CKD-like injury in rats by improving renal function, lipid peroxidation, restoring antioxidant defenses, renal histology and protecting colonic mucosal architecture. The high dose PPE group, among the PPE-treated groups, demonstrated the most comprehensive protective profile and greater numerical improvement than AST-120 in several renal function, oxidative stress, and histopathological endpoints. These findings suggest that PPE may exert nephroprotective activity via multi-target antioxidant, anti-inflammatory, epithelial-protective and gut–kidney axis-related mechanisms. However, no direct inhibition of adenine uptake or 2,8-dihydroxyadenine crystal deposition was shown and requires further mechanistic investigation.

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مستخلص قشر الرمان المعياري يُخفف من مرض الكلى المزمن الناجم عن الأدينين في الفئران: تأثيراته على وظائف الكلى، والإجهاد التأكسدي، والنسيج الكلوي، وبنية القولون

ملخص

يُعرَّف مرض الكلى المزمن (CKD) بتفاقم القصور الكلوي، وتلف بنية الكلى، واضطراب الأكسدة، واضطرابات التمثيل الغذائي الجهازية. يُستخدم إعطاء الأدينين عادةً لمحاكاة خلل وظائف الكلى وإصابة الأنابيب الكلوية في القوارض، كما هو الحال في مرض الكلى المزمن. هدفت هذه الدراسة إلى تقييم فعالية مستخلص قشر الرمان المعياري (PPE) في تخفيف مرض الكلى المزمن الناجم عن الأدينين في الفئران. الطرق: قُسّمت الفئران إلى ست مجموعات: مجموعة الضبط الطبيعية(NC) ، ومجموعة PPE فقط، ومجموعة الضبط المصابة بمرض الكلى المزمن (CKD)، ومجموعة CKD المصابة بمرض الكلى المزمن مع جرعة منخفضة من(PPE (CKD + PPE-L ، ومجموعة CKD المصابة بمرض الكلى المزمن مع جرعة عالية من(PPE (CKD + PPE-H ، ومجموعة CKD المصابة بمرض الكلى المزمن مع العلاج المرجعي AST-120. شملت المعايير التي تم فحصها: الوزن النهائي للجسم، واستهلاك الطعام، ومستوى الكرياتينين في الدم، ومستوى اليوريا في الدم، ومستوى حمض اليوريك في الدم، ومستوى مالونديالدهيد الكلى(MDA) ، ومستوى الجلوتاثيون المختزل(GSH) ، وإنزيم ديسموتاز الفائق(SOD) ، وإنزيم الكاتالاز(CAT) ، وبنية أنسجة الكلى، وبنية القولون. النتائج: أظهرت مجموعة التحكم المصابة بمرض الكلى المزمن ارتفاعًا ملحوظًا في مستويات الكرياتينين واليوريا وحمض اليوريك في الدم، حيث بلغت 2.72 ± 0.35 ملغم/ديسيلتر، و168.4 ± 18.7 ملغم/ديسيلتر، و6.8 ± 0.9 ملغم/ديسيلتر على التوالي، مقارنةً بـ 0.43 ± 0.06 ملغم/ديسيلتر، و37.2 ± 5.1 ملغم/ديسيلتر، و1.3 ± 0.3 ملغم/ديسيلتر لدى مجموعة التحكم السليمة. وقد حسّن تناول جرعات عالية من مستخلص نبات الباوباب هذه الاختلالات، حيث انخفضت مستويات الكرياتينين في الدم إلى 1.31 ± 0.22 ملغم/ديسيلتر، واليوريا إلى 90.6 ± 12.5 ملغم/ديسيلتر، وحمض اليوريك إلى 3.3 ± 0.5 ملغم/ديسيلتر. كما أدى الأدينين إلى زيادة مستوى مالونديالدهيد في الكلى، وانخفاض مستوى الجلوتاثيون، وإنزيم ديسموتاز الفائق، وإنزيم الكاتالاز. أدى تناول جرعات عالية من مستخلص الرمان (PPE) إلى خفض مستوى MDA وتطبيع مستويات GSH و SODو CAT.أظهرت عينات التحكم المصابة بمرض الكلى المزمن (CKD) تلفًا شديدًا في الكلى والقولون، حيث بلغ إجمالي درجات تلف الكلى والقولون 28 و21 على التوالي. وقد خفضت الجرعات العالية من مستخلص الرمان هذه الدرجات بشكل ملحوظ إلى 6 و5 على التوالي. الخلاصة: تشير نتائج هذه الدراسة إلى أن مستخلص الرمان قد يمارس نشاطًا وقائيًا للكلى عبر آليات متعددة الأهداف، تشمل مضادات الأكسدة، ومضادات الالتهاب، وحماية الخلايا الظهارية، وآليات متعلقة بمحور الأمعاء والكلى. ومع ذلك، لم يُلاحظ أي تثبيط مباشر لامتصاص الأدينين أو ترسب بلورات 2،8-ثنائي هيدروكسي أدينين، مما يستدعي إجراء المزيد من الدراسات لفهم الآلية.

الكلمات المفتاحية: الرمان؛ مرض الكلى المزمن؛ الإجهاد التأكسدي؛ علم الأنسجة الكلوية؛ علم الأنسجة القولونية.